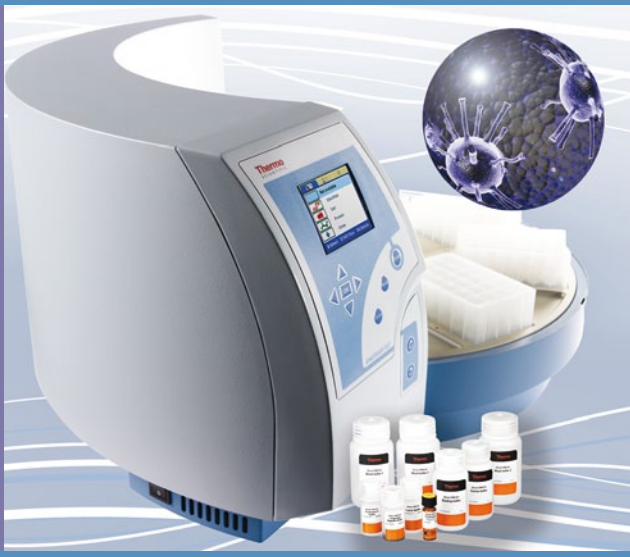
Thermo Scientific KingFisher Viral NA Kit

Instruction Manual

Rev. 1.2



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Thermo Scientific KingFisher Viral NA Kit

Instruction Manual

Rev. 1.2, Cat. no. N11998

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Contents

Chapter 1
Kit Content

Table 1-1. Thermo Scientific KingFisher Viral NA Kit

Item	KingFisher® Viral NA Kit
Cat. No.	97040196
Package size	1 x 96 samples
KingFisher Magnetic Beads	3.2 ml
Proteinase K	1 vial
Proteinase K Buffer	1.8 ml
Carrier RNA	1 vial
Carrier RNA Buffer	500 µl
Lysis Buffer	25 ml
Binding Buffer	65 ml
Wash Buffer 1	55 ml
Wash Buffer 2	55 ml
Wash Buffer 3	65 ml
Elution Buffer	11 ml

The KingFisher Viral NA Kit (Cat. No. 97040196) is intended for the purification of DNA and RNA from samples using the Thermo Scientific KingFisher Flex with a 96 deep well head or the Thermo Scientific KingFisher Duo with a 12-pin head or Thermo Scientific KingFisher mL and a sample volume of 200 µl.

Equipment and reagents to be supplied by the user:

- Magnetic particle processor

Table 1-2. Thermo Scientific KingFisher magnetic particle processors

Cat. No.	Product
5400000	KingFisher magnetic particle processor
5400050	KingFisher mL magnetic particle processor
5400100	KingFisher Duo magnetic particle processor
5400630	KingFisher Flex magnetic particle processor with 96 deep well head
5400640	KingFisher Flex magnetic particle processor with 24 deep well head
Discontinued	KingFisher 96 magnetic particle processor

Table 1-3. Thermo Scientific KingFisher Flex consumables

Cat. No.	Product	Package size
97002514	KingFisher Flex 96 tip comb for PCR magnet	80 pcs
97002524	KingFisher Flex 96 tip comb for KF magnet	100 pcs
97002534	KingFisher Flex 96 tip comb for deep well magnet	100 pcs
97002610	KingFisher Flex 24 deep well tip comb and plate	50 pcs
97002540	KingFisher Flex 96 KF plate (200 µl)	48 pcs
95040450	Microtiter® deep well 96 plate, non sterile	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs

Table 1-4. Thermo Scientific KingFisher Duo consumables

Cat. No.	Product	Package size
97003500	KingFisher Duo 12-tip comb for Microtiter deep well 96 plate	50 pcs
97003510	KingFisher Duo 6-tip combs and KingFisher 24 deep well plate (12 pcs of 24 deep well plates, each including 4 tips combs)	48 pcs
97003520	KingFisher Duo elution strip	40 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates and elution strips for 96 samples)	1 box

Table 1-5. Thermo Scientific KingFisher mL consumables

Cat. No.	Product	Package size
97002111	KingFisher mL tip comb	800 pcs
97002121	KingFisher mL tube	20 x 45 pcs
97002131	KingFisher mL combi (tubes and tip combs for 60 samples)	60
97002141	KingFisher mL combi (tubes and tip combs for 240 samples)	240

Table 1-6. Thermo Scientific KingFisher consumables

Cat. No.	Product	Package size
97002070	KingFisher tip comb	50 pcs
97002080	KingFisher plate 100 µl	50 pcs
97002084	KingFisher plate 200 µl	50 pcs
97002090	KingFisher plastics 100 µl 8-pack, 8 plates and 8 tip combs	1 box
97002094	KingFisher plastics 200 µl 8-pack, 8 plates and 8 tip combs	1 box

Chapter 2
Product Description

Introduction

The KingFisher Viral NA Kit is designed for rapid automated purification of viral RNA and DNA from cell-free body fluids using KingFisher instruments. The nucleic acids (NA) purified using the KingFisher Viral NA Kit are of high quality and free of proteins, nucleases, and other contaminants or inhibitors. They are therefore suitable for direct use in many different downstream applications, such as PCR (polymerase chain reaction), restriction analysis and in several other enzymatic reactions.

Intended use

The KingFisher Viral NA Kit is developed for the purification of viral RNA and DNA from cell-free body fluids, such as serum or plasma, using paramagnetic particles. The purification process requires no phenol/chloroform extraction or alcohol precipitation and needs very little hands-on time. The reagents and specific plastic consumables are designed to work with the KingFisher Flex, KingFisher Duo or KingFisher mL magnetic particle processors as part of an integrated system. The KingFisher Viral NA Kit is only intended for research use, not for clinical or diagnostic use. The user is responsible for validating the performance of the KingFisher instrument and the KingFisher Viral NA Kit for any particular use, because the performance of the kits has not been validated for any specific organism.

Principle and procedure

The KingFisher Viral NA Kit uses magnetic-particle technology for viral RNA and DNA purification. The Thermo Scientific KingFisher technology combines the speed and efficiency of nucleic acid purification with easy handling of magnetic particles. Nucleic acids bind to the surface of the Thermo Scientific KingFisher Magnetic

Beads in the presence of a chaotropic salt. The following effective wash steps dispose of proteins, cell debris and any residual contaminants, while the nucleic acids bound to the KingFisher Magnetic Beads are transferred through sequential wash steps. Three different Wash Buffers are used, followed by an air drying step, which considerably improves the purity of the nucleic acids. High-quality nucleic acids are eluted into the Elution Buffer and are ready for subsequent downstream processes, such as enzymatic reactions. Heat incubation is included in the lysis and elution steps of the purification protocol when using the KingFisher Flex or KingFisher Duo.

Kit specifications

The KingFisher Viral NA Kit is designed for rapid automated preparation of highly pure viral nucleic acids from cell-free body fluids using Thermo Scientific KingFisher magnetic particle processors. When excluding a dispense step requiring the addition of Binding Buffer and KingFisher Magnetic Beads, the approximate processing time is 40 minutes for the purification of 96 samples in the KingFisher Flex and 12 samples in the KingFisher Duo, and 45 minutes for the purification of 15 samples in the KingFisher mL. The obtained nucleic acids can be used directly in various downstream applications.

Suitable sample materials for purification are cell-free body fluids, such as serum and plasma. The procedure is optimized for the sample volume of 200 µl. The sample type as well as the handling and storage of the sample affect the yield of the purified nucleic acids.

The KingFisher Viral NA Kit can be processed at room temperature, but to ensure a good yield of purified nucleic acids, it is advisable to use heating during the lysis and elution steps or in case of the KingFisher mL, heat the Lysis and Elution Buffers.

The KingFisher Magnetic Beads are highly reactive, superparamagnetic beads.

KingFisher magnetic particle processors

The KingFisher magnetic particle processors are designed for the automated transfer and processing of magnetic particles in microplate format. The patented technology of the Thermo Scientific KingFisher systems is based on the use of magnetic rods covered with disposable, specially designed tip combs and plates or tubes. Use only Thermo Scientific KingFisher plastic consumables. Use of products from other manufacturers may cause unsuitable mixing or even instability in the KingFisher instrument. The instrument functions without any dispensing or aspiration parts or devices. Samples and reagents, including magnetic particles, are dispensed into the plates according to the corresponding instructions. Dispensing can be done manually or partially automatically using automatic dispensers, for example, the Thermo Scientific Multidrop Combi and/or the Thermo Scientific Versette. Thermo Scientific BindIt Software 3.2 can be used for running ready-made and optimized protocols for the Thermo Scientific KingFisher Kits. It is also possible to transfer the defined protocol onto the onboard software and run it directly from the instrument. The KingFisher instruments offer a rapid and automated solution for complicated and time-consuming purification processes without risk of carryover or cross contamination, resulting in high-purity nucleic acids.

The KingFisher instrument family comprises four systems covering working volumes from 20 to 5000 µl. Each system consists of an instrument, specially designed plastic consumables and the easy-to-use BindIt® Software 3.2. The KingFisher Viral NA Kit is optimized and ready for use with KingFisher instruments.

The KingFisher magnetic particle processors are intended for professional research use by trained personnel. Detailed information and user instructions for the KingFisher instruments can be found in their respective user manuals.

Table 2-1. Overview of Thermo Scientific KingFisher systems

	KingFisher Flex		KingFisher Duo	
	96 format	**24 format**	**12 format**	**6 format**
Processing volume	20–1000 µl	200–5000 µl	30–1000 µl	200–5000 µl
Capacity	Up to 96 samples per run (sample volume 200 µl with the KingFisher Viral NA Kit)	Up to 24 samples per run	Up to 12 samples per run	Up to 6 samples per run
Magnetic head	96 interchangeable formats for PCR plate, KingFisher Flex 96 KF plate, Microtiter deep well 96 plate	24 format for KingFisher Flex 24 deep well plate	12-pin magnet head for Microtiter deep well 96 plate	6-pin magnet head for KingFisher Flex 24 deep well plate
Plates	KingFisher Flex 96 KF plate (20–200 µl), 96 well PCR plate, skirted (20–100 µl), Microtiter deep well 96 plate (50–1000 µl)	KingFisher Flex 24 deep well plate (200–5000 µl)	Microtiter deep well 96 plate (50–1000 µl), KingFisher Duo elution strip (30–130 µl)	KingFisher Flex 24 deep well plate (200–5000 µl)
Tip combs	KingFisher Flex 96 tip comb for PCR magnets, KingFisher Flex tip comb for KF magnets, KingFisher Flex 96 tip comb for deep well magnets	KingFisher Flex 24 tip comb for deep well magnets	KingFisher Duo 12-tip comb	KingFisher Duo 6-tip comb
Heating temperature	Heating block temperature from +5°C above ambient room temperature to +115°C		Heating block temperature from +10°C to +75°C, elution strip +4°C to +75°C in room temperature	

Table 2-1. Overview of Thermo Scientific KingFisher systems

	KingFisher mL	KingFisher
Processing volume	50–1000 µl	20–200 µl
Capacity	Up to 15 samples per run (sample volume 200 µl with the KingFisher Viral NA Kit)	Up to 24 samples per run
Magnetic head	15 format	24 format
Plates	KingFisher mL tube, special tube strip with 1 x 5 tubes (50–1000 µl)	KingFisher plate 100 or 200 µl (20–100 µl or 20–200 µl)
Tip combs	KingFisher mL tip comb, 1 x 5 format	KingFisher tip comb, 1 x 12 format
Heating temperature	No heating available	No heating available

The BindIt Software 3.2 protocols optimized for the KingFisher Viral NA Kit are available for the KingFisher Flex, the KingFisher Duo and the KingFisher mL instruments. BindIt Software 3.2 protocols for the Thermo Scientific KingFisher and the Thermo Scientific KingFisher 96 are available on request. For more information, contact your local authorized distributor.

Chapter 3
Safety Information

The following components of the KingFisher Viral NA Kit contain hazardous contents (Table 3-1).

Wear a laboratory coat, disposable gloves and goggles, and follow the safety instructions given in the kit instruction manual. It is recommended that Good Laboratory Practice (GLP) is followed to guarantee reliable analyses.

Table 3-1. Safety precautions

Reagent	Hazardous contents	Safety instructions
Lysis Buffer	Guanidine thiocyanate < 50%	Harmful by inhalation, in contact with skin and if swallowed. Contact with acids liberates very toxic gas. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Keep away from food, drink and animal feed.
Binding Buffer	Sodium perchlorate < 30% and ethanol < 55%	Flammable. Harmful if swallowed.
Wash Buffer 1	Sodium perchlorate < 18% and ethanol < 24%	Flammable.
Wash Buffer 2	Ethanol < 80%	Highly flammable. Keep container tightly closed. Keep away from sources of ignition.
Carrier RNA Buffer	Guanidine thiocyanate < 50%	Harmful by inhalation, in contact with skin and if swallowed. Contact with acids liberates very toxic gas. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Keep away from food, drink and animal feed.
Proteinase K	Lyophilized Proteinase K	Irritating to eyes, respiratory system and skin. May cause sensitization by inhalation. Do not inhale dust. Avoid contact with skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Chapter 4

Storage Conditions and Preparation of the Working Solutions

Storage conditions

All buffers and reagents included in the KingFisher Viral NA Kit can be stored at room temperature (20–25°C) and are stable for up to one year from the manufacturing date. The buffers are ready for use.

Preparation of the Proteinase K working solution

To prepare the Proteinase K working solution for the KingFisher Viral NA Kit, add 1 ml of Proteinase K Buffer to the vial of the lyophilized Proteinase K.

The Proteinase K working solution should be stored at -20°C in aliquots. Repeated freezing and thawing should be avoided.

Preparation of the Carrier RNA working solution

To prepare the Carrier RNA working solution, add 500 µl of Carrier RNA Buffer to the vial of the Carrier RNA.

The Carrier RNA working solution should be stored at -20°C in aliquots. Repeated freezing and thawing should be avoided.

Chapter 5
Protocols and Pipetting Instructions

Before beginning the nucleic acid purification protocol, carefully read through the *Thermo Scientific KingFisher Flex User Manual* (Cat. No. N07669), the *Thermo Scientific KingFisher Duo User Manual* (Cat. No. N12420) or the *Thermo Scientific KingFisher mL User Manual* (Cat. No. 1508260), and the *Thermo Scientific BindIt Software for KingFisher Instruments version 3.2 User Manual* (Cat. No. N07974).

BindIt Software 3.2 protocols for the KingFisher and the KingFisher 96 can be obtained on request.

Handling of KingFisher Magnetic Beads

A homogeneous distribution of the KingFisher Magnetic Beads in the container is essential before the beads are transferred to the wells or tubes in order to ensure a high consistency between the wells or tubes. To gain complete resuspension of the beads, shake the container vigorously or vortex briefly. The KingFisher Magnetic Beads have a tendency to sediment relatively quickly in the Binding Buffer. Once a premixture of the beads and the Binding Buffer has been made, the mixture should be used immediately to avoid the risk of transferring variable amounts of the beads to the respective wells or tubes.

Instructions for KingFisher Flex with 96 deep well plates for nucleic acid purification from 200 µl of cell-free body fluid

These instructions are for the nucleic acid purification from 200 µl of cell-free body fluid using the KingFisher Viral NA Kit (Cat. No. 97040196) and the KingFisher Flex with Thermo Scientific Microtiter deep well 96 plates.

When using the KingFisher Viral NA Kit for the first time, prepare the working solutions for Proteinase K and Carrier RNA. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Working Solutions".

1. Take four empty Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher Flex 96 KF plates.

2. Prepare four Microtiter deep well 96 plates and one KingFisher Flex 96 KF plate as indicated in the table below.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Sample	200 µl
			Lysis Buffer	200 µl
			Proteinase K working solution	10 µl
			Carrier RNA working solution	4 µl
2	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1	500 µl
3	Microtiter deep well 96 plate	Wash 2	Wash Buffer 2	500 µl
4	Microtiter deep well 96 plate	Wash 3	Wash Buffer 3	550 µl
5	KingFisher Flex 96 KF plate	Elution	Elution Buffer	100 µl

3. Place a Thermo Scientific KingFisher Flex 96 tip comb for deep well magnets on a **Tip Plate** (that is, an empty KingFisher Flex 96 KF plate).

4. Start the KF_ViralNA_Flex96 protocol with the KingFisher Flex 96 and load the plates.

Switch on the KingFisher Flex and make sure that you are using the KingFisher Flex 96 deep well head and heating block. Connect the PC with BindIt Software 3.2 to the KingFisher Flex. Start the KF_ViralNA_Flex96 protocol. Insert the Tip Plate and the filled plates into the instrument as indicated on the KingFisher Flex display. After all the plates have been loaded into the instrument, the protocol will begin.

When the KingFisher Flex is to be run as a standalone instrument, transfer the KF_ViralNA_Flex96 protocol to the KingFisher Flex. The instructions for transferring the protocol can be found in Chapter 4: "*Using the software*" in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

5. Add the KingFisher Magnetic Beads and Binding Buffer to the Sample plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the lysis step (approximately 15 minutes after starting the protocol run), remove the Sample plate from the instrument and separately add the KingFisher Magnetic Beads and Binding Buffer to the **Sample plate** as indicated below. Resuspend the KingFisher Magnetic Beads well (e.g., by vortexing) before transferring them from the bottle.

Plate number	Plate type	Plate name	Content	Reagent volume per well
1	Microtiter deep well 96 plate	Sample	KingFisher Magnetic Beads	30 µl
			Binding Buffer	550 µl

6. Place the Sample plate back into the instrument and press **Start**. After the pause, the protocol will continue to the end.

7. After the run is completed, remove the plates and store the purified nucleic acids.

When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and turn off the instrument. Store the purified nucleic acids accordingly. The purified nucleic acids are ready for use in downstream applications.

The final nucleic acid concentration in the Elution Buffer may increase if the purified nucleic acids are eluted into a smaller volume of the buffer than is recommended, but this can slightly reduce the overall nucleic acid yield.

Summary of plate contents

Table 5-1. Summary of plate contents

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Sample	200 µl
			Lysis Buffer	200 µl
			Proteinase K working solution	10 µl
			Carrier RNA working solution	4 µl
Dispense step: add 30 µl of KingFisher Magnetic Beads per well and 550 µl of Binding Buffer per well to the Sample plate (Plate 1) during the pause in the BindIt protocol.				
2	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1	500 µl
3	Microtiter deep well 96 plate	Wash 2	Wash Buffer 2	500 µl
4	Microtiter deep well 96 plate	Wash 3	Wash Buffer 3	550 µl
5	KingFisher Flex 96 KF plate	Elution	Elution Buffer	100 µl
6	KingFisher Flex 96 KF plate	Tip Plate		

Instructions for KingFisher Duo with 12-pin magnet head and 96 deep well plates for nucleic acid purification from 200 µl of cell-free body fluids

These instructions are for the nucleic acid purification from 200 µl of cell-free body fluid using the KingFisher Viral NA Kit (Cat. No. 97040196) and the KingFisher Duo with a 12-pin magnet head and Microtiter deep well 96 plates.

When using the KingFisher Viral NA Kit for the first time, prepare the working solutions for Proteinase K and Carrier RNA. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Working Solutions".

1. Take one empty Microtiter deep well 96 plate and one Thermo Scientific KingFisher Duo elution strip.

2. Prepare the **Viral NA plate** (Microtiter deep well 96 plate).

Add the following reagents to the rows. **Note that row B is reserved for the tip comb and should be left empty. Note that rows C, D and E are left empty.**

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Viral NA plate	A	Sample	Sample	200 µl
Microtiter deep well 96 plate			Lysis Buffer	200 µl
			Proteinase K working solution	10 µl
			Carrier RNA working solution	4 µl
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Empty	Empty	Empty
	F	Wash 1	Wash Buffer 1	500 µl
	G	Wash 2	Wash Buffer 2	500 µl
	H	Wash 3	Wash Buffer 3	550 µl

3. Fill the KingFisher Duo elution strip as follows. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the Elution Buffer is pipetted into the correct wells.

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Elution Buffer	100 µl

4. Place a Thermo Scientific KingFisher Duo 12-tip comb into row B on a Viral NA plate.

5. Start the KF_viralNA_Duo protocol with the KingFisher Duo and load the plate and elution strip.

Switch on the KingFisher Duo and make sure that you are using the KingFisher Duo 12-pin magnet head and heating block. Connect the PC with BindIt Software 3.2 to the KingFisher Duo. Start the KF_viralNA_Duo protocol. Insert the Viral NA plate and elution strip into the instrument as indicated on the KingFisher Duo display and press **OK**. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user.

When the KingFisher Duo is to be run as a standalone instrument, transfer the KF_viralNA_Duo protocol to the KingFisher Duo. The instructions for transferring the protocol can be found in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and Binding Buffer to row A during the dispense step.

When the KingFisher Duo pauses at the dispense step after the lysis step (approximately 15 minutes after starting

the protocol run), remove the Viral NA plate from the instrument and separately add the KingFisher Magnetic Beads and Binding Buffer to row A in the Viral NA plate as indicated below. Resuspend the KingFisher Magnetic Beads well (e.g., by vortexing) before transferring them from the bottle.

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Viral NA plate	A	Sample	KingFisher Magnetic Beads	30 µl
Microtiter deep well 96 plate			Binding Buffer	550 µl

7. Place the Viral NA plate back into the instrument and press **OK**. After the pause, the protocol will continue to the end.

8. After the run is completed, remove the plate and store the purified nucleic acids.

When the protocol is completed, remove the plate and elution strip according to the instructions on the KingFisher Duo display and turn off the instrument. Store the purified nucleic acids accordingly. The purified nucleic acids are ready for use in downstream applications. The final nucleic acid concentration in the Elution Buffer may increase if the purified nucleic acids are eluted into a smaller volume of the buffer than is recommended, but this can slightly reduce the overall nucleic acid yield.

Summary of plate and elution strip contents

Table 5-2. Summary of plate and elution strip contents

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Viral NA plate	A	Sample	Sample	200 µl
Microtiter deep well 96 plate			Lysis Buffer	200 µl
			Proteinase K working solution	10 µl
			Carrier RNA working solution	4 µl
	Dispense step: add 30 µl of KingFisher Magnetic Beads and 550 µl of Binding Buffer per well to row A during the pause in the BindIt protocol.			
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Empty	Empty	Empty
	F	Wash 1	Wash Buffer 1	500 µl
	G	Wash 2	Wash Buffer 2	500 µl
	H	Wash 3	Wash Buffer 3	550 µl
Elution strip		Elution	Elution Buffer	100 µl

Instructions for KingFisher mL for nucleic acid purification from 200 µl of cell-free body fluid

These instructions are for the nucleic acid purification from 200 µl of cell-free body fluid using the KingFisher Viral NA Kit (Cat. No. 97040196) and the KingFisher mL.

When using the KingFisher Viral NA Kit for the first time, prepare the working solutions for Proteinase K and Carrier RNA. For more instructions, refer to Chapter 4: "Storage Conditions and Preparation of the Working Solutions".

A tube strip tray in the KingFisher mL may contain up to 15 separate Thermo Scientific KingFisher mL tube strips, and one sample processing uses one tube strip with five tubes. The orientation of the tube strip is fixed. Note that the tube strips have to be positioned so that the slip ends are facing left. One tip comb with five tips is used to process five samples at a time.

1. Place empty KingFisher mL tubes on a tube strip tray.

2. Prepare the **tubes** (that is, starting from the first tube at the slip end of a tube strip). Add the following reagents to the tubes.

Tube	Tube name	Content	Reagent volume
A	Sample	Sample	200 µl
		Lysis Buffer	200 µl
		Proteinase K working solution	10 µl
		Carrier RNA working solution	4 µl
B	Wash 1	Wash Buffer 1	500 µl
C	Wash 2	Wash Buffer 2	500 µl
D	Wash 3	Wash Buffer 3	550 µl
E	Elution	Elution Buffer	100 µl

3. Prepare the KingFisher mL for the run.

Switch on the KingFisher mL and insert the tray into the instrument. Insert the tip combs into their slots and close the front lid.

4. Start the KF_ViralNA_mL protocol with the KingFisher mL.

Connect the PC with BindIt Software 3.2 to the KingFisher mL. Start the KF_ViralNA_mL protocol.

When the KingFisher mL is to be run as a standalone instrument, transfer the KF_ViralNA_mL protocol to the KingFisher mL. The instructions for transferring the protocol can be found in Chapter 4: "*Using the software*" in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

5. Add the KingFisher Magnetic Beads and Binding Buffer to the Sample tubes during the dispense step.

When the KingFisher mL pauses at the dispense step after the lysis step (approximately after 20 minutes), remove the tube strip tray from the instrument and add the KingFisher Magnetic Beads and Binding Buffer separately to the **Sample tubes** as indicated below. Resuspend the KingFisher Magnetic Beads well (e.g., by vortexing) before removing them from the bottle.

Tube	Tube name	Content	Reagent volume
A	Sample	KingFisher Magnetic Beads	30 µl
		Binding Buffer	550 µl

6. Place the tube strip tray back into the instrument and press **Start**. After the pause, the protocol will continue to the end.

7. After the run is completed, remove the tube strips and store the purified nucleic acids.

When the protocol is completed, remove the tubes and turn off the instrument. Store the purified nucleic acids accordingly. The purified nucleic acids are ready for use in downstream applications.

To increase the nucleic acid yield, the Lysis Buffer can be prewarmed to 55°C and the Sample tubes can be prepared just before the beginning of the purification protocol. The Elution Buffer can also be prewarmed to 55°C and dispensed into the Elution tubes during an additional pause step in the protocol before the elution step.

If some KingFisher Magnetic Beads remain in the elution, place the tubes on a magnet for a few minutes to collect the residual beads at the bottom of the tube. The final nucleic acid concentration in the Elution Buffer may increase if the purified nucleic acids are eluted into a smaller volume of the buffer than is recommended, but this can slightly reduce the overall nucleic acid yield.

Summary of tube contents

Table 5-3. Summary of tube contents

Tube	Tube name	Content	Sample/reagent volume
A	Sample	Sample	200 µl
		Lysis Buffer	200 µl
		Proteinase K working solution	10 µl
		Carrier RNA working solution	4 µl
Dispense step: add 30 µl of KingFisher Magnetic Beads per tube and 550 µl of Binding Buffer per tube into the Sample tubes (A tubes) during the pause in the BindIt protocol.			
B	Wash 1	Wash Buffer 1	500 µl
C	Wash 2	Wash Buffer 2	500 µl
D	Wash 3	Wash Buffer 3	550 µl
E	Elution	Elution Buffer	100 µl

Quantification of purified viral RNA and DNA

The amount of viral nucleic acids in cell-free body fluids is usually very small and the yields of purified nucleic acids are low. In addition, the Carrier RNA is co-purified and affects the analyses of the yield with a spectrophotometer. To determine the yield of viral nucleic acids, it is recommended to use amplification techniques.

Chapter 6
General Information

Reagent specificity and volumes

A reagent must not be used with any kit other than that for which it is intended. It is strongly recommended that the volume of reagents in each well or tube is kept within the limits specified in the *KingFisher Flex User Manual*, *KingFisher Duo User Manual* or *KingFisher mL User Manual* to avoid spillover and to keep performance at the most efficient level.

Handling of magnetic beads

The KingFisher Magnetic Beads have a tendency to sediment relatively quickly in the Binding Buffer. Once a premixture of the beads and Binding Buffer has been made, the mixture should be used immediately to avoid the risk of transferring variable amounts of the beads to the respective wells or tubes. The amount of beads in the wells or tubes affects the yield of the purified nucleic acids.

Binding and wash steps

The binding between the nucleic acids and the KingFisher Magnetic Beads is strong in the presence of a chaotropic salt, but chaotropic salts are not present in the Wash Buffer 3 and accordingly the binding is weak. Avoid strong mixing speeds and releasing the KingFisher Magnetic Beads into the Wash Buffer 3 in order to minimize the loss of nucleic acids during the wash step. A short wash and a slow mixing speed without releasing the beads into the buffer are recommended.

Elution step

Carryover of ethanol to the Elution Buffer causes impurities in the Elution Buffer and may affect some downstream applications. To remove traces of ethanol, make sure that there is a wash step (e.g., washing without releasing the beads) or the drying step before the elution step is long enough. There is a delicate balance between complete removal of the ethanol and loss of nucleic acids.

The volume of the Elution Buffer can be modified depending on the user requirements concerning the purified nucleic acid concentration. The final nucleic acid concentration in the Elution Buffer may increase if the purified nucleic acids are eluted into a smaller volume of the buffer, but this can slightly reduce the overall nucleic acid yield. The modifications of the elution step must be done in BindIt Software 3.2 and according to the volume ranges suitable for the KingFisher instrument. The table below indicates the available elution volumes of the KingFisher instruments.

Table 6-1. Available elution volumes of Thermo Scientific KingFisher instruments

KingFisher instrument	Elution volume
KingFisher	20–200 µl
KingFisher mL	50–1000 µl
KingFisher Duo with 12-pin magnet head	30–130 µl
KingFisher Duo with 6-pin magnet head	200–5000 µl
KingFisher Flex with 96 deep well head, elution in a KingFisher Flex 96 KF plate	50–150 µl
KingFisher Flex with 96 deep well head, elution in a Microtiter deep well 96 plate	50–1000 µl
KingFisher Flex with 96 well head, elution in a KingFisher Flex 96 KF plate	20–250 µl
KingFisher Flex with 24 deep well head	200–5000 µl

To gain a maximal yield of purified nucleic acids, avoid the lowest permitted volumes of Elution Buffer in the KingFisher instruments. The Elution Buffer should cover the KingFisher Magnetic Beads completely and

any possible magnetic-bead pellet should be completely resuspended. In addition, the volume of the Elution Buffer should be adequate for efficient mixing of the beads in order to obtain a maximal release of the purified nucleic acids from the beads.

Elution can be conducted at room temperature. However, it is strongly recommended that both the lysis and elution steps are carried out at 56°C in the KingFisher Flex and KingFisher Duo in accordance with the BindIt Software 3.2 protocol. When using the KingFisher instrument without a heating option, the Lysis and Elution Buffers can be prewarmed to 55°C and dispensed into the Lysis or Elution tubes. The Elution Buffer should be dispensed into the Elution tubes during an additional pause in the protocol before the elution step.

If some KingFisher Magnetic Beads remain in the Elution Buffer, centrifuge the Elution plate briefly or place the Elution plate or the tube strip on a magnet for a few minutes to collect the residual beads at the bottom of the well or tube and transfer the supernatant to a new tube.

Decontamination and disinfection of sample material

The sample material and the reagents and plastics that have been in contact with the sample material should be decontaminated in order to minimize the risk of contamination. For this purpose, a decontaminant, such as Virkon®, should be used in accordance with the manufacturer's instructions. Accordingly, the appropriate treatment and/or disposal of waste should be taken care of.

Appendix A
Troubleshooting

Table A-1. Troubleshooting guide

Problem	Possible cause and actions
Low DNA yield	There should be an adequate volume of the Elution Buffer to cover the KingFisher Magnetic Beads completely during the elution step. Do not let the KingFisher Magnetic Beads dry, as this might result in lower elution efficiency. Efficient lysis of the sample material at 56°C or preheating the Lysis Buffer to 55°C increases the nucleic acid yield. Heating during the elution step or using a preheated Elution Buffer enhances the release of the nucleic acids from the KingFisher Magnetic Beads and the yield of the purified nucleic acids. Prolonged storage of the sample material may reduce the nucleic acid yield. Use only Thermo Scientific KingFisher plates or tubes with the KingFisher instruments. Use of products from other manufacturers may cause unsuitable mixing and affect the yield of purified nucleic acids.
Low purity	Prolonged storage of the sample material may reduce the quality of the nucleic acids. Insufficient washing causes impurities in the Elution Buffer. Wash Buffers 1 and 2 contain ethanol. Carryover of the buffers may cause unsatisfactory performance in downstream applications. Carryover of the KingFisher Magnetic Beads to the Elution Buffer may affect the A_{260}/A_{280} ratio. Make sure that the KingFisher Magnetic Beads do not affect the measurement by centrifuging the samples or placing them on a magnet for a few minutes to collect the residual beads at the bottom of the well. Carryover of the KingFisher Magnetic Beads does not affect most downstream processes.

Continued

Continued

Problem	Possible cause and actions
Magnetic particles remaining in the lysed sample or elution well	Starting material that is too viscose prevents efficient collection of the KingFisher Magnetic Beads from the lysed sample. The magnetic rods will not be able to collect all the beads unless the viscose samples are diluted before the beginning of the purification process. The samples can, for example, be diluted into 1 x PBS. Improper lysis may also cause problems collecting the KingFisher Magnetic Beads. If the KingFisher Magnetic Beads are inefficiently collected from the Elution Buffer, the addition of a small amount of detergent (e.g., 0.02% Tween 20) may improve the results. Carryover of the KingFisher Magnetic Beads to the Elution Buffer may affect the A_{260}/A_{280} ratio. Refer to "Low purity" on page 39. KingFisher Magnetic Beads that occasionally remain attached to the tip combs at the end of the process do not affect the nucleic acid yield, as the nucleic acids have already been released from the KingFisher Magnetic Beads into the Elution Buffer. If the KingFisher magnetic particle processor does not work properly, refer to the relevant user manual of the KingFisher instrument in use.

Appendix B
Ordering Information

Table B-1. Thermo Scientific KingFisher Viral NA Kits

Cat. No.	Product	Package size
97040196	KingFisher Viral NA Kit	1 x 96

Table B-2. Thermo Scientific KingFisher Flex consumables

Cat. No.	Product	Package size
97002514	KingFisher Flex 96 tip comb for PCR magnet	80 pcs
97002524	KingFisher Flex 96 tip comb for KF magnet	100 pcs
97002534	KingFisher Flex 96 tip comb for deep well magnet	100 pcs
97002610	KingFisher Flex 24 deep well tip comb and plate	50 pcs
97002540	KingFisher Flex 96 KF plate (200 µl)	48 pcs
95040450	Microtiter deep well 96 plate, non sterile	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs

Table B-3. Thermo Scientific KingFisher Duo consumables

Cat. No.	Product	Package size
97003500	KingFisher Duo 12-tip comb for Microtiter deep well 96 plate	50 pcs
97003510	KingFisher Duo 6-tip combs and KingFisher 24 deep well plate (12 pcs of 24 deep well plates, each including 4 tips combs)	48 pcs
97003520	KingFisher Duo elution strip	40 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates and elution strips for 96 samples)	1 box

Table B-4. Thermo Scientific KingFisher mL consumables

Cat. No.	Product	Package size
97002111	KingFisher mL tip comb	800 pcs
97002121	KingFisher mL tube	20 x 45 pcs
97002131	KingFisher mL combi (tubes and tip combs for 60 samples)	60
97002141	KingFisher mL combi (tubes and tip combs for 240 samples)	240

Table B-5. Thermo Scientific KingFisher consumables

Cat. No.	Product	Package size
97002070	KingFisher tip comb	50 pcs
97002080	KingFisher plate 100 µl	50 pcs
97002084	KingFisher plate 200 µl	50 pcs
97002090	KingFisher plastics 100 µl 8-pack, 8 plates and 8 tip combs	1 box
97002094	KingFisher plastics 200 µl 8-pack, 8 plates and 8 tip combs	1 box

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